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Food sources of two detritivore amphipods associated with the seagrass *Posidonia oceanica* leaf litter

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Abstract

This study focused on the ingestion and assimilation of *Posidonia oceanica* (L.) Delile litter by *Gammarella fucicola* Leach and *Gammarus aequicauda* Martynov, two dominant detritivore amphipods of the *P. oceanica* leaf litter. Scanning electron microscope observations indicated that leaf litter is highly colonized by diverse diatoms, bacteria and fungi, which may constitute a potential food source for the litter fauna. Gut content observations demonstrated that these species eat *P. oceanica* litter, and that this item is an important part of their ingested diet. Stable isotope analyses showed that the species do not experience the same gains from the ingested *Posidonia*. *Gammarella fucicola* displayed isotopic values, suggesting a major contribution of algal material (micro- and macro-epiphytes or drift macro-algae). On the other hand, the observed isotopic values of *G. aequicauda* indicated a more important contribution of *P. oceanica* carbon. The mixing model used agreed with this view, with a mean contribution of *P. oceanica* to approximately 50% (range 40-55%) of the assimilated biomass of *G. aequicauda.* This demonstrated that the two species, suspected to be detritus feeders, display in reality relatively different diets, showing that a certain degree of trophic diversity may exist among the detritivore community of the seagrass litter.

Key words: Mediterranean Sea ; mixing model ; phytodetritus ; seagrass ; stable isotopes

Introduction

The coastal oceans play a significant role in the global carbon cycle (e.g. Borges 2005). Macro-phytes, and among them seagrasses, are key actors of carbon metabolism in these areas (e.g. Gattuso et al. 1998; Duarte et al. 2005). The fate of seagrass primary production is variable depending on the system studied. Recent work has shown that herbiv-ory may be an important trophic route in seagrass beds (Valentine & Heck 1999) but, perhaps due to the anthropogenic decimation of large seagrass herbivores (Heck & Valentine 2006), the detrital trophic pathway seems prominent (and even dominant) in most seagrass beds (Duarte & Cebrian 1996). Despite its importance, the nature and controls of the detrital trophic web in seagrass communities are not yet fully understood. This is a much needed step towards improving our still incomplete knowledge of how seagrass communities function and their roles in coastal ecosystems, which in turn may also be helpful for the design of policies of ecologically sustainable human coastal development.

Posidonia oceanica (L.) Delile is a large seagrass species endemic to the Mediterranean, forming a climax association of sand substrate. In this seagrass system, pioneer papers and those that followed have shown that the detritus pathway (i.e. decomposition, exportation, storage) is dominant in the energy flow, although this is very variable according to meadow characteristics or location (Ott & Maurer 1977; Romero et al. 1992; Pergent et al. 1994, 1997; Mateo & Romero 1997; Cebrian & Duarte 2001; Danovaro et al. 2002). The *P. oceanica* leaf detritus resulting from natural leaf fall accumulates as litter on sandy areas adjacent to the seagrass meadow or within the seagrass bed on smaller sand patches. According to Walker et al. (2001), "true" litter refers to recognizable dead leaf fragments in-mixing with entire dead leaves lying on the sediment after their abscission. Uprooted *P oceanica* and drift macro-algae from the adjacent rocks are often found in-mixing with this "true litter". In *P. oceanica* beds, leaf fall occurs throughout the year, following a physiologically controlled process of senescing, but the leaf fall rate increases dramatically during the autumn. Litter accumulation is maximal during this period. Nevertheless, litter accumulations may be found throughout the year.

The *Posidonia* litter shelters an abundant animal community and many micro-organisms or algae (bacteria, marine fungi, protozoa, micro-algae). Animal diversity in the litter is high, but is lower than in the meadow foliar stratum (Gambi et al. 1992; Gallmetzer et al. 2005). Leaf stratum fauna is mostly herbivore or deposit feeder (Gambi et al. 1992), although some species eat *Posidonia* detritus as a secondary food source (Mazzella et al. 1992). On the other hand, detritivores are thought to be the dominant species of leaf litter (Gallmetzer et al. 2005). Wittman et al. (1981) have shown in the laboratory that amphipods feeding on dead *Posidonia* leaves accelerate dramatically the fragmentation of material and increase its degradation rate. Mateo & Romero (1996)

demonstrated that in situ the mechanical fragmentation of leaf material caused by physical and biological factors accelerates the decomposition of *P. oceanica* detritus. This had also been demonstrated by an earlier study by Fenchel (1970) in a *Thalassia testudinum* ecosystem.

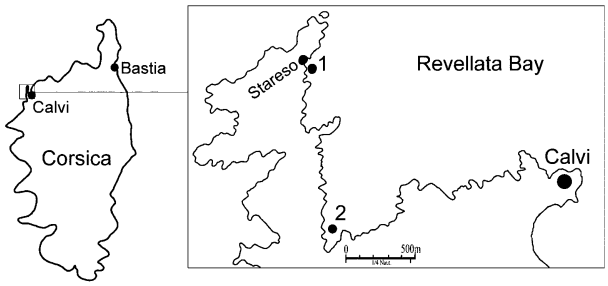
Fauna could also contribute to this degradation by assimilating directly seagrass material, shortcutting the longer detritus food chain that often implies the export of leaf detritus. This shortcut could be important for ecosystem processes by constituting a way to maintain seagrass primary production in the bed itself or in a closely adjacent area. The only way to infer the assimilation of seagrass material by the litter fauna is to use trophic tracers, because stomach contents inform only on the nature of ingested food and not on the nature of assimilated materials.

In order to determine the role of two amphipods in seagrass litter consumption and assimilation, we observed their gut contents and used their carbon and nitrogen isotopic ratios as trophic tracers.

Study area

Sample collection was undertaken at two different sites at Revellata Bay (Gulf of Calvi, Occidental Corsican coast, northwest Mediterranean) (8°45'E 42°35'N) (Figure 1), near the oceanographic station STARESO (University of Liège). The seagrass *P. oceanica* covers approximately 50% of the Revellata Bay sea bottom, reaching a depth of 40 m. The meadow of the Gulf of Calvi is among the most productive *P. oceanica* beds in the northwest Mediterranean (Pergent-Martini et al. 1994), despite the oligotrophic character of coastal Corsican waters (Gobert et al. 2002).

Figure 1. *The location of sampling sites in Revellata Bay (Gulf of Calvi, Corsica) (1, STARESO; 2, Alga Bay).*



In front of STARESO (Station 1), the *P. oceanica* bed is continuously reaching a mean density of 450 shoots m^{-2} at 10 m depth (Gobert et al. 2003). The leaf litter is found lying on small sand patches (1-10 m^2) in or close to the seagrass bed. The slope of the rocky shore close to this site does not allow the accumulation of seagrass wrack (i.e. *Posidonia* "banquettes"). The second collection site is a fringing and shallow (i.e. between 5 and 10 m depth) seagrass bed along the rocky shore of Alga Bay. The centre of Alga Bay is composed of fine sandy sediment and is colonized by patches of the seagrass *Cymodocea nodosa.* This large central sand patch (i.e. approximately 2.6 hectares) is an accumulation zone for seagrass leaf litter, which forms aggregates of approximately 10 m^2 along the fringing *P. oceanica* bed, floating a few centimetres above the sediment. The head of Alga Bay is a beach where *Posidonia* wracks accumulate as "banquettes".

Material and methods

Litter is defined in this study as the material lying on the sediment, composed of abscised dead *Posidonia* leaves, degraded but recognizable *Posidonia* leaf fragments, uprooted living *Posidonia* shoots and also drift macro-algae. It was observed at Stations 1 and 2 (Figure 1) in March 2004 at a depth of between 5 and 8 m. Drift macro-algae grow on adjacent rocks or are epiphytes of *Posidonia* rhizomes. The brown algae *Halopteris* spp. and *Dictyota* spp. were seen to dominate, but *Cystoseira* spp. (brown algae), *Udotea petiolata* (green algae), *Sphaerococus* sp. (red algae) and erect corallines (calcareous red algae) were also found. Random collection was repeatedly carried out in different accumulation zones for each investigated site (n = 5 patches per site), a few metres apart. Scuba divers placed all the material constituting litter inside large plastic bags (approximately 50 1) per patch and closed them to limit fauna escape. The samples of litter were carefully sorted in order to collect amphipods. Two amphipod species largely dominated the litter fauna in terms of individual numbers (approximately >80% of litter fauna).

Identifications were made under stereoscopic microscope (Ruffo 1982, 1998). Dissections were also carried out under stereoscopic microscope for gut content observation of individuals belonging to the two dominant amphipod species *Gammarus aequicauda* Martynov and *Gammarella fucicola* Leach. Gut content observations were made under optic microscope in order to detect the presence of *Posidonia* material for 10 adults of each species having the same size. Analysed individuals were collected from Alga Bay where the two species co-occurred.

Samples for isotopic and elemental measurements were oven dried for at least 48 h at 50°C and were ground to a homogenous powder using pestle and mortar. Measurements of carbon and nitrogen isotopic ratios and elemental contents were made on primary producer material and on the two dominant amphipods found in the litter. Analysed primary producers were dead *Posidonia* leaves (n = 4 random pools from the two locations), drift macro-algae species (n = 10 species; some individuals were pooled per measurement) found in the litter and epiphytes (both algae and fixed animal) (n = 4 random pools from the two locations) present on the dead *Posidonia* fragments. The *P. oceanica* fragments were scraped with a razor blade under a binocular microscope to remove epiphytes before analysis.

Measurements were made on a single individual adult specimen of *G. aequicauda* (n = 30 individuals, sorted into the following groups: 15 adult females and 15 adult males). For adults of *G. fucicola* and juveniles of both species, the amount of organic matter was insufficient to perform such individual analyses and three to five individuals were pooled (n = 17 pools sorted into the following groups: five adult *G. fucicola* females, five adult *G. fucicola* males, four *G. aequicauda* juveniles and three *G. fucicola* juveniles).

After grinding, samples that contained inorganic carbonates (i.e. amphipods, epiphytes, calcified algae) were acidified with HC1 (1N). Measurements were performed with a mass spectrometer coupled to a C-N-S elemental analyser for combustion and automated analysis. As recommended by Pinnegar & Polunin (1999), for samples to be acidified, $^{15}N/^{14}N$ ratios were measured before acidification due to significant modifications of $^{15}N/^{14}N$ after HC1 addition. Ratios are presented as δ values (‰), expressed relative to the vPDB (Vienna Peedee Belemnite) standard and to atmospheric N_2 for carbon and nitrogen, respectively. Reference materials were IAEA-N1 ($\delta^{15}N = +0.4\pm0.2$‰) and IAEA CH-6 (sucrose) ($\delta^{13}C = -10.4\pm0.2$‰). The standard deviation on replicates of a *Posidonia* pool was 0.3 and 0.4‰ for carbon and nitrogen, respectively.

The results of isotopic measurements were used in a mixing model to calculate the potential contribution of three food sources (i.e. *Posidonia* litter, sciaphilous drift algae, and an aggregated food source composed of drift photophilous algae and epiphytes). These three food sources were distinguishable by their $\delta^{13}C$ signature but only weakly by their $\delta^{15}N$ values. This increased the uncertainty of the mixing model results. For this reason, potential food source contributions are presented as mean values (%) associated to a range of possible contributions (Phillips & Gregg 2003).

The basic mixing model was formulated as:

$$\delta_m{}^1 = f_a\delta_a{}^1 + f_b\delta_b{}^1 + f_c\delta_c{}^1$$
$$\delta_m{}^2 = f_a\delta_a{}^2 + f_b\delta_b{}^2 + f_c\delta_c{}^2$$
$$1 = f_a + f_b + f_c$$

where δl and δ2 represent the isotopic signatures of two different elements (i.e. carbon and nitrogen in this study) used to calculate the partition of the contribution (f) of three food sources (a, b, c) to a mixture (m). δm is given by:

$$\delta_m = \delta_{animal} - \Delta$$

where Δ is the isotopic enrichment factor between animal tissue and its diet. This Δ is the net result of different isotopic fractionations (i.e. preferential use of one isotope over another) occurring during nitrogen metabolism of animals. This isotopic enrichment for nitrogen ($\Delta^{15}N$) is variable but generally positive (i.e. it tends to increase $\delta^{15}N$ of animal against its food). We used the method of Phillips & Gregg (2001, 2003) and Philips et al. (2005) to resolve these equations with their free software (www.epa.gov/wed/pages/models/isotopes/ isosource.htm). Models were run with an enrichment factor for ^{15}N between animals and potential diet equal to 0, +0.5, +1, +2, +3‰. Enrichments of +2 and +3‰ in ^{15}N did not yield any solutions. We finally chose the enrichment values leading to the best constrained mixing model solutions. For *G. fucicola,* enrichment was set to 0‰ and for *G. aequicauda* to 1‰. These values are in the range reported by Vanderklift & Ponsard (2003) for detritivore diets. Contributions were generated with an increment of 1%. We used a tolerance level of 0.2‰.

In May 2005, litter pieces were individually hand picked by divers at the two locations and placed in sterile vials. Material for scanning electron microscopy was fixed within 1 h in 4% seawater glutar-aldehyde (0.2 µm pre-filtered) at 4°C (three times 24 hours), gently rinsed in filtered seawater and post-fixed in 1 % OsO_4 in mQ water for 1 h. Samples were prepared for scanning electron microscopy, according to the method of Dauby & Poulicek (1995). Material for epifluorescence microscopy was fixed in 2% formaldehyde dissolved in 0.2 µm filtered seawater. It was treated just before examination by adding 0.1 ‰ acridine orange (final concentration) to the samples (incubation 3 min), rinsed five times for 2 min in mQ water, and then embedded in oil of cedar wood.

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Results

All states of degradation (i.e. green leaf, senescing leaf, fresh abscised, degraded or highly degraded material) were found at the two locations. Nevertheless, the litter from Alga Bay was mainly composed of large pieces of dead *P. oceanica* leaves and was less altered than the litter collected in front of STARESO, where the litter was very fragmented. Leaf fragments were sometimes colonized by macro-epiphytes (mainly red coralline algae and bryozo-ans). *Cymodocea nodosa* detritus was almost absent from Alga litter accumulation, despite the fact that a sparse meadow of this species exists in this area.

The *P. oceanica* litter was heavily colonized by diatoms *(Coconeis* spp.), bacteria (rod, vibrionid, coccoid-shaped and filamentous) and marine filamentous unseptate fungi (Figures 2 and 3). This colonization occurred mainly at the surface of leaf fragments, but some leaf pieces were more degraded and showed growing and ramifying fungi or bacteria inside the tissues, as shown by epifluorescence microscopy (Figure 4). A few cyanobacteria were also recorded by epifluorescence microscopy. Where the fragments were more degraded, the *Posidonia* cells had been emptied of their initial constituents and were sometimes filled by diatoms and bacteria. Some pieces of litter were obviously parts of faecal pellets (remains of peritrophic membranes, dead fragmented diatoms, embedded within a heavy mucoid coat, larger bacteria).

Figure 2. *Scanning electron microscope observations of leaf fragments from the Posidonia litter sampled in May 2005 in Revellata Bay (Gulf of Calvi, Corsica). (A) A fragment of a green leaf relatively undegraded, showing the presence of many diatoms and a diffuse bacteria coverage. (B) A senescing leaf showing large bacterial colonies, filamentous bacteria and diatoms. Note the disappearing of cuticles in the bottom left-hand corner. (C) Details of (B) showing a bacterial colony. (D) A degraded dead leaf fragment, showing dense coverage of diatoms and numerous fungi.*

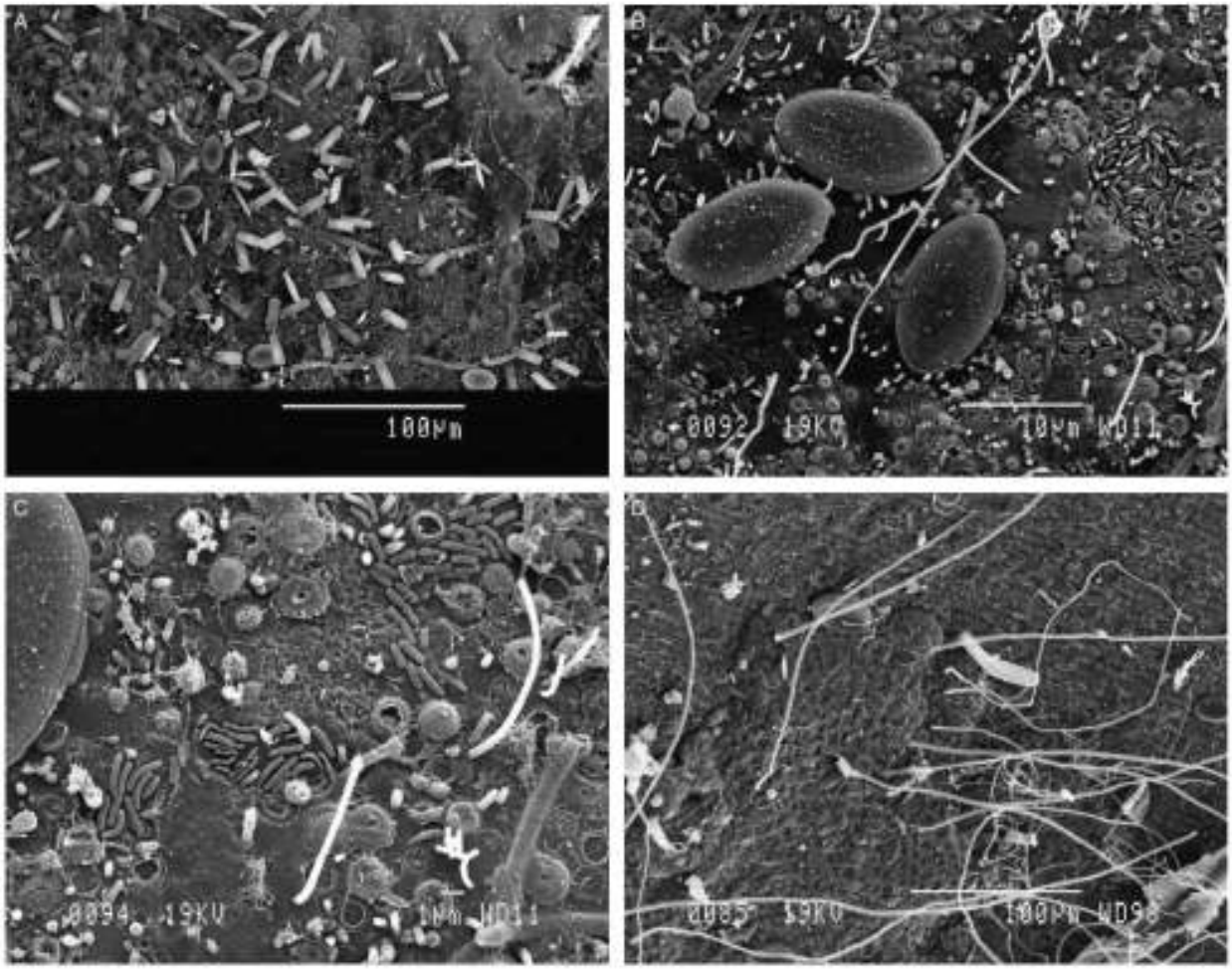

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***Figure 3**. Scanning electron microscope observations of leaf fragments from the Posidonia litter sampled in May 2005 in Revellata Bay (Gulf of Calvi, Corsica). (A) Degraded leaf litter (a semi-transparent dead leaf fragment), showing the presence of diatoms and fungi in germination. (B) Detail of (A) showing the great density of diatom pavement, diatoms inside the emptied cells, and the presence of a dense bacteria coverage (some morphological types). (C) Small fragments of very degraded litter (digested particles?) showing diatom remains and probably mucus film. (D) Details of (C) showing diatom remains.*

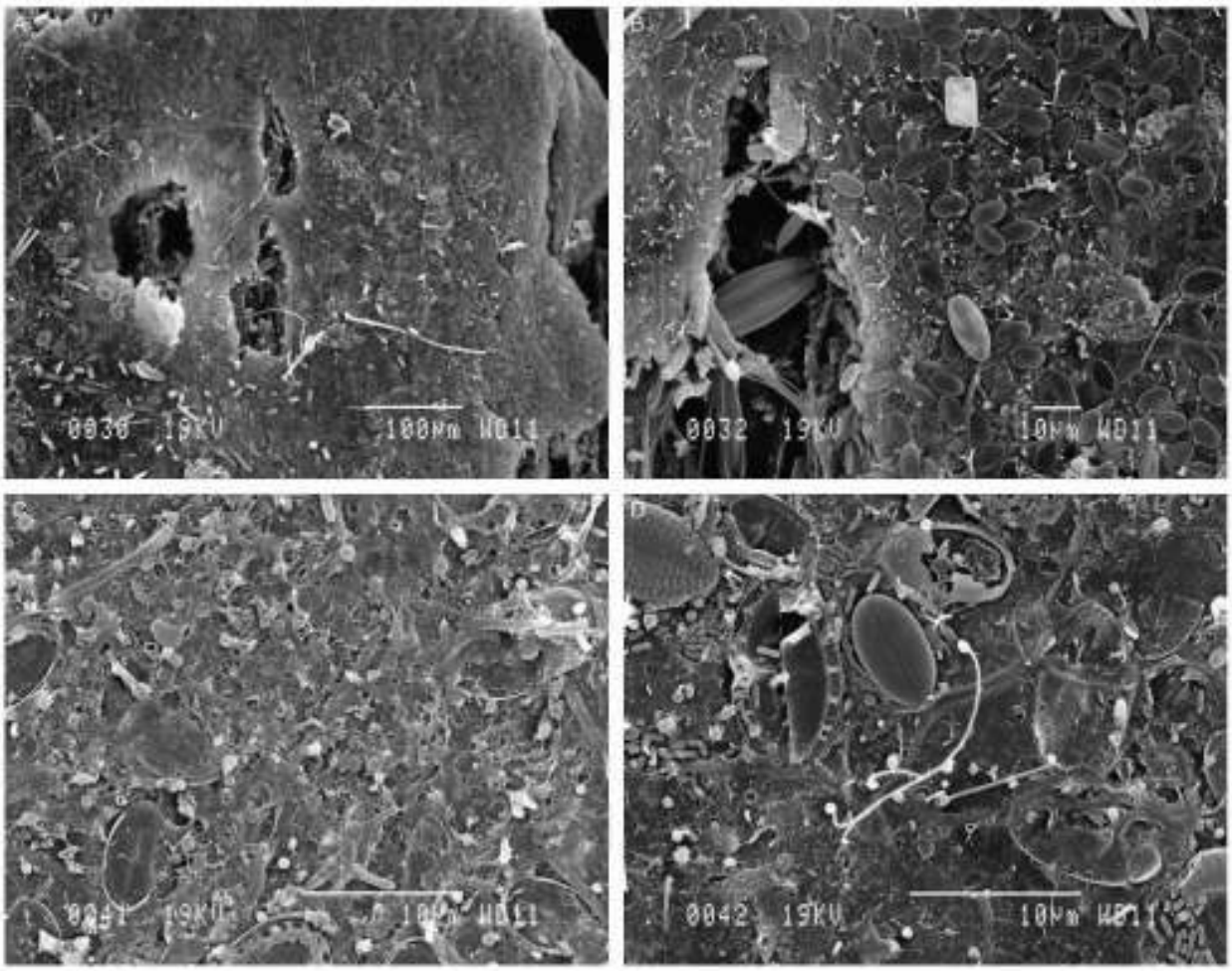
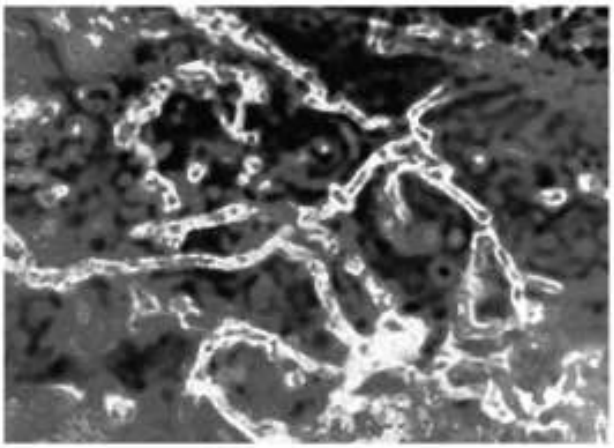


***Figure 4.** Epifluorescence photography of a degraded leaf litter fragment of Posidonia oceanica sampled in May 2005 in Revellata Bay (Gulf of Calvi, Corsica) showing the presence of marine fungi inside the dead leaf, and not viewable with classical scanning electron microscope observation.*



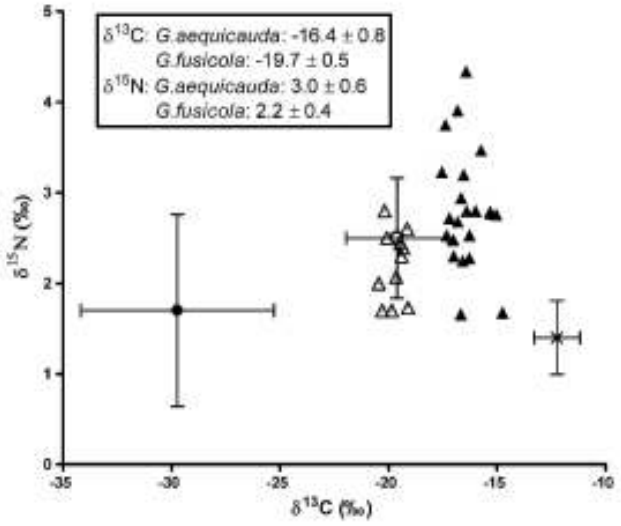
The dominant amphipod at Alga was *G. aequi-cauda,* which displayed sizes of greater than 1 cm. However, in front of STARESO, it was *G. fucicola* that displayed sizes of less than 1 cm. *Gammarella fucicola* was also present at Alga, but *G. aequicauda* was absent from the STARESO litter. Both species had eaten dead *Posidonia* material in significant quantities. Fragments were small (5-100 cells) and were degraded (i.e. often emptied of their internal material). All individuals of *G. aequicauda* displayed seagrass litter in their gut. The occurrence of *Posidonia* pieces was important in *G. aequicauda,* reaching almost 100% of examined gut content. Fragments of epiphytic algae (mainly soft erect algae) or of other plant material were also found in small amounts. Some green pieces of *P. oceanica* were observed in one individual of *G. aequicauda.*

Approximately 50% of *G. fucicola* individuals had ingested dead *Posidonia.* The contribution of *Posidonia* material was less important in *G. fucicola,* where the most important item was unidentified algae. Micro-organisms (foraminifera, diatoms) were also present in the gut of *G. fucicola.*

Small unidentified animals were present in the gut contents of 20% of individuals of the two species.

The $\delta^{13}C$ values of dead *Posidonia* material varied between -0.7 and -13.5‰ (Figure 5). These were significantly less negative values than for the epiphytes and drift algae found in the litter (Mann-Whitney test, $P < 0.001$). $\delta^{13}C$ values ranged from -17.3 to -19.5‰ for *P. oceanica* epiphytes and from -16.8 to -35.6‰ for drift macro-algae. Drift macro-algae belong to two ecological groups (i.e. algae from adjacent photophilous biota and sciaphi-lous algae growing in unexposed zones or on *P. oceanica* rhizomes; Janssens et al. 1993). Averaged (± standard deviation) $\delta^{13}C$ values were -19.8 ± 2.3‰ for algae of photophilous biota and -29.7 ± 4.5‰ for more sciaphilous algae, and differed significantly (Mann-Whitney, $P < 0.0001$). These differences allowed the aggregation of data into three potential food sources for mixing model calculations: *Posidonia* litter (-12.2 ± 1.1), epiphytes and drift photophilous algae (-19.6 ± 2.3) and sciaphilous drift algae (-29.7 ± 4.5‰).

Figure 5. *$\delta^{13}C$ and $\delta^{15}N$ of Gammarus aequicauda (black triangle) and Gammarella fucicola (open triangle) sampled in the Posidonia oceanica litter accumulated in the Gulf of Calvi and of their potential food sources (mean ± standard deviation) (●: drift sciaphilous algae, O: complex of drift photophilous algae plus epiphytes of P. oceanica, x: Posidonia litter).*



The $\delta^{15}N$ values of leaf litter ranged from 0.9 to 1.7‰. Drift macro-algae and epiphytes displayed values ranging from 0.9 to 5.4‰ and from 0.9 to 2.3‰, respectively. Due to the low nitrogen content of leaf detritus and epiphytes, it was impossible to obtain $\delta^{15}N$ from all samples. As a result, the number of replicates was insufficient to perform a statistical analysis on $\delta^{15}N$ of litter and epiphytes ($n = 3$). Average values introduced in the mixing model for potential food source were 1.4, 2.5, 1.8‰ for *P. oceanica,* the aggregation of drift photophilous algae and epiphytes, and drift sciaphilous algae, respectively.

Gammarus aequicauda displayed δ C values between -14.7 and -17.5‰. The $\delta^{13}C$ values observed for *G. fucicola* differed significantly from those of *G. aequicauda* (Kolmogorov-Smirnov, $P < 0.001$) and were between -19.1 and -20.5‰.

The $\delta^{15}N$ values of *G. aequicauda* and *G. fucicola* varied between 1.7 and 4.3‰ and between 1.7 and 2.7‰, respectively, and were significantly different (Kolmogorov-Smirnov, $P < 0.005$).

On average (± standard deviation) (range of possible solutions), in the *G. aequicauda* diet, *P. oceanica* litter represented 50 ± 4% (45-55). The complex photophilous algae plus epiphytes represented 44 ± 6% (35-50) and

drift sciaphilous algae 6 ± 3% (0-10) (Figure 6). Contributions of these sources to the diet of *G. fucicola* were 20 ± 7% (6-30), 70 ± 12% (50-90) and 10 ± 5% (0-20), respectively (Figure 7).

Figure 6. *Dietary contributions of three potential food sources for the gammarid crustacean Gammarus aequicauda. The contributions were calculated for all mixing model iterations (in 1% increments) and are expressed as a percentage frequency of all possible solutions. The mean contribution of each source is labelled.*

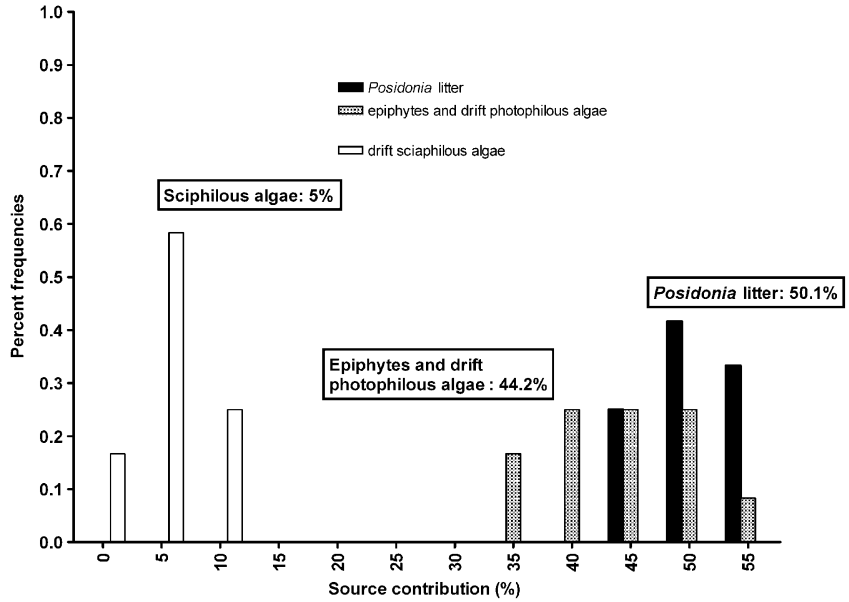
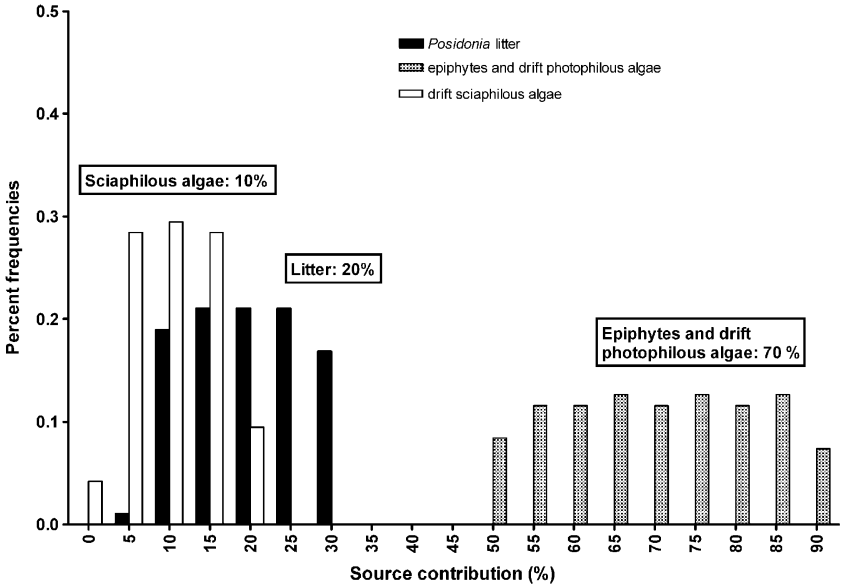


Figure 7. *Dietary contributions of three potential food sources for the gammarid crustacean Gammarella fucicola. The contributions were calculated for all mixing model iterations (in 1% increments) and are expressed as a percentage frequency of all possible solutions. The mean contribution of each source is labelled.*



Discussion

The gut contents of *G. aequicauda* and *G. fucicola* confirm that these species ingest a significant quantity of *P. oceanica* litter. This item is not anecdotic in their diet and is particularly important in the diet of *G. aequicauda* (i.e. almost all guts were filled with *P. oceanica* particles). Litter pieces found in the guts of *G. aequicauda* and *G. fucicola* were of relatively small size and seemed deeply affected by digestion. This underlines the potential role of these species in the mechanical degradation of *P. oceanica* detritus.

The two amphipod species had significantly different $\delta^{13}C$ values, reflecting the difference in their assimilated food. The application of the mixing model of Philips & Gregg (2003) generated all possible contributions of three distinct food sources to the diet of the two species. The solutions shown in Figures 6 and 7 confirm these

differences, in spite of model uncertainties due to the weakness of ^{15}N differences between sources, as underlined by Vizzini & Mazzola (2003) or Pinnegar & Polunin (2000). *Gammarella fucicola* appears to preferentially assimilate algal carbon coming from photophilous drift algae and micro- and *macro-Posidonia* epiphytes. On the other hand, in the *G. aequicauda* diet, *Posidonia* litter could represent almost half of the carbon assimilated by the animal. Despite its uncertainties, the mixing model is a useful tool, which allows the detection of a particular food source. Indeed, this model indicated that *Posidonia* litter is potentially present in the *G. fucicola* diet, although it probably constitutes a small part of the assimilated carbon. This contrasts with the amphipods from the foliar stratum, which displayed $\delta^{13}C$ values lower (-20.1‰) than those of litter fauna amphipods, indicating a preferential use of algal carbon in the foliar stratum (aggregated species, dominated by *Apherusa* spp.) (Lepoint et al. 2000). This confirms the distinct trophic characteristics of the two amphipod communities (i.e. vagile fauna of foliar stratum versus litter fauna) (Gambi et al. 1992 versus Gallmetzer et al. 2005).

The $\delta^{15}N$ values of the two amphipods were relatively low and were close to the $\delta^{15}N$ values of primary producers. It is often observed that the $\delta^{15}N$ of animals increases with increasing trophic levels (e.g. Post 2002). This isotopic enrichment ($\Delta^{15}N$) is the net result of different isotopic fractionations (i.e. preferential use of one isotope over another) occurring during animal nitrogen metabolism. It is generalized that this increase is close to 3‰ between two consecutive trophic levels (e.g. Post 2002). Nevertheless, this enrichment may vary according to trophic group, to phylum, to excretion characteristics or to food quality (e.g. Vander Zanden & Rasmussen 2001; McCutchan et al. 2003; Adams & Sterner 2000; Vanderklift & Ponsard 2003). Vanderklift & Ponsard (2003) pointed out that detritivore crustacean have often very low $\Delta^{15}N$ against their food (<1‰). This matches our observation of $\delta^{15}N$ values for *G. fucicola* being close to the $\delta^{15}N$ values of primary producers, and lower than for carnivorous invertebrates (Lepoint et al. 2000). *Gammarus aequicauda* displayed $\delta^{15}N$ values significantly higher than those of *G. fucicola,* although its gut contents clearly indicated a detriti-vore diet. We imposed a greater fractionation factor (l‰) to encompass this difference. Nevertheless, this could be an indication of another nitrogen source with higher $\delta^{15}N$ values than those of the primary producers analysed. This alternative food source could consist of the micro-epiphytes living at the surface of leaf litter, which appear as very abundant on the leaf surface (Figures 2 and 3), but the source could also be the animal items found in some gut contents of *G. aequicauda* in small quantities. In this case, the $\delta^{15}N$ signature of *G. aequicauda* resulted from the mixing of ^{15}N signatures of different food sources (litter, micro-organisms, animals) and from a low fractionation factor characteristic of detritivore diet. The use of another trophic tracer, such as a sulphur isotope (Connolly et al. 2004), a fatty acid biomarker or an experimental assessment of isotopic fractionation could provide more information on this question.

Many females and males were found paired for both species, and juveniles were observed in female marsupium or as free individuals, showing that the two species are able to accomplish their whole biological cycle in the *Posidonia* litter. Therefore, this habitat not only provides a shelter for associated fauna, but also constitutes an appropriate food source, at least for the typical fauna of the *Posidonia* litter (i.e. detritivore amphipods, leptostracean and *Idoteidae* isopods). However, the question of the digestibility capacity of detritivore fauna is still in debate with regard to many ecosystems. This question arises from the fact that angiosperm detritus is refractory to digestion (i.e. it has a high content of structural carbohydrate and high C:N ratios).

Seagrass detritus, and particularly *P. oceanica,* has a high content of lignin, which is also rather refractory to bacterial digestion (Klap et al. 2000). The nitrogen content of *Posidonia* detritus is lower than in living leaves because nitrogen is resorbed in senescing leaves before abscission and because labile nitrogen is quickly mobilized by bacterial degradation (Mateo & Romero 1997). It is generally thought that almost all the nitrogen present in the leaf detritus after a few weeks is associated with a refractory component or that it has a microbial origin (bacterial and/or fungal origin) (e.g. Newell 1996).

Nevertheless, our isotopic data demonstrate a significant assimilation by *G. aequicauda* and, probably by *G. fucicola*, *P. oceanica* carbon, despite its apparent low digestibility and low food quality. This assimilation could be explained by direct or indirect digestion pathways. On the one hand, the amphipods have the capacity to digest the structural carbon of leaf detritus, or, on the other hand, this assimilation is mediated through microbes and fungi biomass. This last hypothesis could only be true if the isotopic signature of bacteria and fungi is relatively close to the signature of their substrate (i.e. *Posidonia* detritus) . In other words, the assimilation of *P. oceanica* material by fungi or bacteria should not involve any carbon fractionation of the *Posidonia* isotopic ratio during this transfer. Indeed, such fractionation would give a less negative $\delta^{13}C$ value to this food source, and therefore to the amphipod ^{13}C signature. This absence (or reduction) of fractionation between *Posidonia* detritus and bacterial carbon is observed in bacteria associated with pristine *Posidonia* bed sediment (Holmer et al. 2004). To our knowledge, such measurements do not exist for marine fungi associated with detritus. However, there is a substantial enrichment in ^{13}C between decaying wood (or leaf litter) and terrestrial saprophytic fungal carbon (approximately +3.5‰) (Hobbie et al. 2001). If this enrichment is transposable to marine fungi, this

could indicate that fungal carbon is not a dominant food source of *G. aequicauda,* considering the isotopic values measured for this species. Nevertheless, the associated fungal community could be important, either by making digestible compounds more accessible to feeders, as demonstrated for talitrid amphipod *Uhlorchestia spartinophila* in *Spartina alterniflora* saltmarsh (Kneib et al. 1997), or as a substantial nitrogen source. Indeed, terrestrial saprophytic fungi display relatively low C:N ratios (between 9 and 15) and high nitrogen concentrations (3-6%) (Taylor et al. 2003).

The alternative hypothesis to a microbial mediation for seagrass carbon assimilation would be that the amphipods possess the capacity to digest by themselves the refractory component of their food. This capacity may be due to the production of enzymes or to an association with endosymbiotic bacteria. Endosymbiotic associations are demonstrated for some aquatic or semi-terrestrial species of detritivore isopods, such as *Asellus aquaticus* or *Ligia pallasii,* but apparently not in marine isopods belonging to the *Idotea* genus (Zimmer et al. 2002; Zimmer & Bartholomé 2003). In amphipods, it seems that endosymbiosis is not the rule (Zimmer & Bartholomé 2003). On the other hand, some authors have demonstrated the production by aquatic amphipods or isopods of endocellulases, endox-ylanases, phenol oxidases or endolaminarinases (McGrath & Matthews 2000; Zimmer et al. 2002; Johnston et al. 2005). As pointed out by Zimmer & Bartholomé (2003), direct assimilation and mediation by micro-epiphytes may coexist in the same species, allowing this species to meet its nutritive needs and to accomplish its entire biological cycle in very special habitats, such as angiosperm litter.

The dominant species in the litter fauna appears to be able to assimilate a part of the ingested *Posidonia* carbon and in this way to constitute a link between seagrass primary production and adjacent habitats. Indeed, the fauna developing in this litter is consumed by local shore fishes. This link between adjacent habitats would be a feature shared with other seagrass ecosystems. In southern Australia, this link between seagrass production and the commercially important fish *Sillago schomburgkii* is mediated through detritivore polychaete worms inhabiting mudflats adjacent to or within the seagrass meadows (Connolly et al. 2005). Along the Californian coast, in detritus mats formed by *Macro-cystis* and the seagrass *Phyllospadix* sp., detritivore amphipods and leptostracean crustaceans form a trophic link to predatory fishes (Vetter 1998). In southwestern Australia, *Amphibolis* sp. and *Posidonia* spp. detritus form large accumulations in-mixing with drift macro-algae. Although it is not the main food source of the fauna inhabiting these accumulations, species of harpacticoids copepods, amphipods or polychaetes assimilate effectively seagrass material (Hyndes & Lavery 2005).

This preliminary study was not sufficient to quantify the role of detritivore fauna in seagrass litter decomposition, but it showed that the two potential means of action (i.e. mechanical fragmentation and direct assimilation) occur in the litter accumulation. Moreover, this study demonstrated that, although these two amphipods are true detri-tivores, they do not have the same diet in terms of carbon sources. This enhances the importance of the biodiversity aspect of this special and transient habitat, one which is generally ignored by protection or management policy or in food web models (Moore et al. 2004).

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